Application of an HPLC method for the quality control of vitamin C content in foods for infants

Mafalda A. Silva[a], Tânia Gonçalves Albuquerque[a,b], M. Beatriz P. P. Oliveira[b], Helena S. Costa[a,b*]

[a] Department of Food and Nutrition, National Institute of Health Dr. Ricardo Jorge, I.P., Av. Padre Cruz 1649-016 Lisbon, Portugal
[b] REQUIMTE-LAQV/Faculdade de Farmácia da Universidade do Porto, Rua de Jorge Viterbo Ferreira n.º 228, 4050-313 Porto, Portugal
*helena.costa@insa.min-saude.pt

Vitamin C is an important water-soluble vitamin that humans are not able to synthesize, so it needs to be provided by the diet.

Regulation (EU) No. 609/2013, Commission Delegated Regulation (EU) No. 2016/127 and *Ministério da Agricultura, do Desenvolvimento Rural e das Pescas* (2008) establishes compositional and information requirements for foodstuffs for infants and young children [1-3].

Babies less than 1 year usually do not eat foods naturally rich in vitamin C, so the main sources are breast milk and/or infant formulas.

Adequate intake of vitamin C is very important to assure children's good health and development, so it is crucial to evaluate vitamin C content in this type of foods.

Aim

The aim of this study was to validate a high-performance liquid chromatography (HPLC) method for the quality control of vitamin C content in infant foods (2 infant formulae (IF), 2 follow-on formulae (FF), 2 processed cereal-based foods (PCF) and 2 baby foods (BF)).

Materials and Methods

Sampling

In 2016, the infant foods were collected from major supermarket chains and parapharmacies in the region of Lisbon (Portugal).

Two of the acquired samples were already ready-to-use, while the remaining samples were prepared according to manufacturer's instructions.

Sample extraction and chromatographic analysis were performed according to Valente *et al*. (2014) and Silva *et al*. (2017) [4,5].

6 scoops of an infant formula powder + **180 mL of boiled water** = **Infant formula**

Results and Discussion

The HPLC-DAD analytical method was validated for selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy, using an infant formula, according to international guidelines [6,7].

Figure 1 shows HPLC chromatograms for L-ascorbic acid standard (A) and for sample BF2 from baby foods' category.

Calibration curves were linear over the range 1-100 µg/mL. The achieved LOD and LOQ were 0.026 and 0.086 µg/mL, respectively (Table 1).

The repeatability RSD (n=6) for vitamin C ranged from 0.54% to 1.85%. The intermediate precision RSD (n=18) was 4.63%, 7.64 ± 0.35 µg/mL (Table 2).

Vitamin C content in the analysed samples ranged from 1.5 ± 0.01 to 178 ± 1.01 mg/100 g for BF1 and PCF2, respectively (Figure 2).

Table 1. Linearity, LOD and LOQ for vitamin C determination in infant foods.

Concentration range (µg/mL)		1 - 100
Slope (n=6)	Mean ± SD	$9.58 \times 10^4 \pm 1.59 \times 10^3$
	RSD (%)	1.66
Intercept (n=6)	Mean ± SD	$7.28 \times 10^4 \pm 1.98 \times 10^4$
	RSD (%)	27.23
Determination coefficient (r^2)	Mean ± SD	0.9996 ± 0.00
	RSD (%)	0.03
Equipment repeatability	RSD (%)	0.07
LOD (µg/mL)		0.026
LOQ (µg/mL)		0.086

Table 2. Validation data for method precision.

Precision		Vitamin C	
		Mean (µg/mL) ± SD	RSD (%)
Repeatability	Day 1 (n=6)	7.48 ± 0.04	0.54
	Day 2 (n=6)	7.34 ± 0.14	1.85
	Day 3 (n=6)	8.10 ± 0.09	1.13
Intermediate precision (n=18)		7.64 ± 0.35	4.63

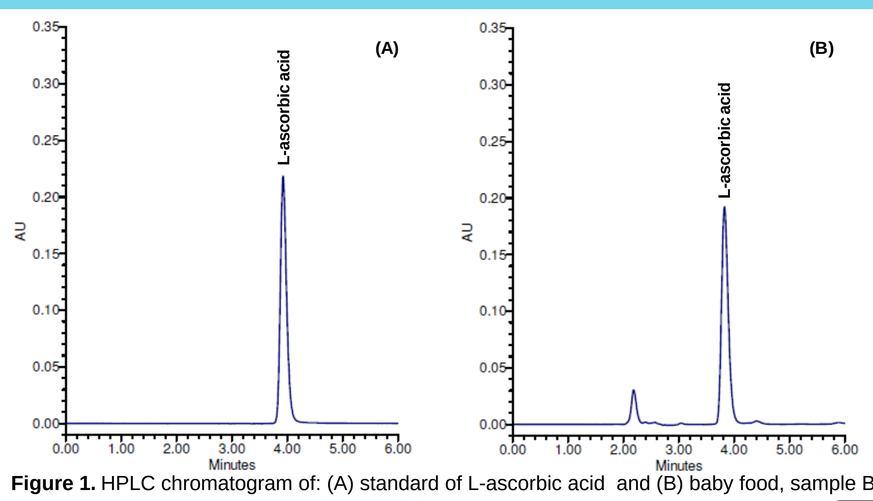

Figure 1. HPLC chromatogram of: (A) standard of L-ascorbic acid and (B) baby food, sample BF2.

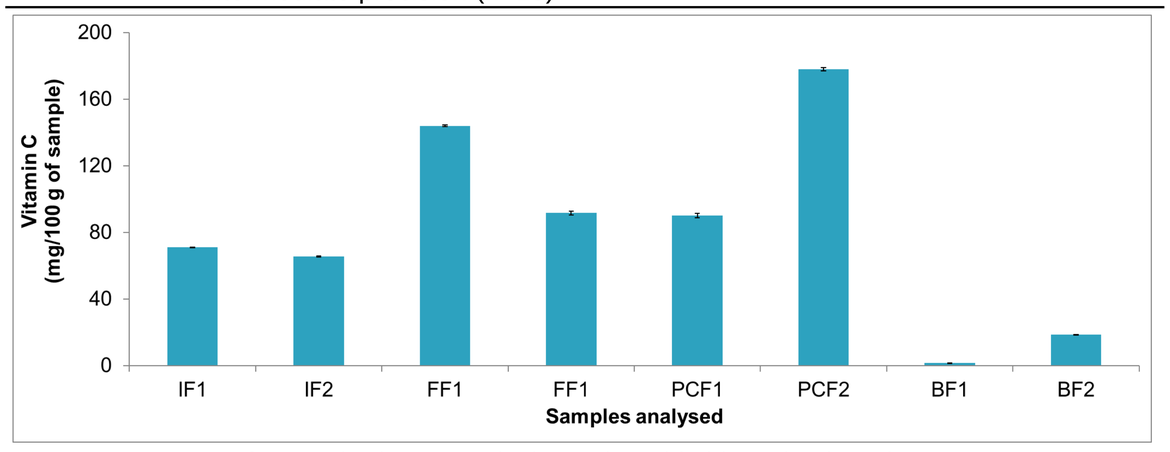

Figure 2. Vitamin C content (mg/100 g of sample) of the analysed samples.
IF – Infant formula; FF – Follow-on formulae; PCF – Processed cereal-based foods; BF – Baby foods

Conclusions

The developed method is rapid, specific, precise and accurate, for the quantification of vitamin C in different categories of foods for infants and young children, showing satisfactory data for all the tested parameters. Since these type of food products are very important for such young and vulnerable consumers, regulation and assurance of an adequate intake of essential nutrients, like vitamin C, is extremely important for a healthy development.

References

[1] European Parliament/Council of the European Union. (2013). Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control. Official Journal of the European Union, L181, 35-56.
[2] European Commission. (2016). Commission Delegated Regulation (EU) 2016/127 of 25 September 2015 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information requirements for infant formula and follow-on formula. Official Journal of the European Union, L25, 1-29.
[3] Ministério da Agricultura, do Desenvolvimento Rural e das Pescas (2008). Decreto-Lei n.º 53/2008 de 25 de Março. Diário da República, 1ª série - Nº 59, 1667-1674.
[4] Valente, A., Sanches-Silva, A., Albuquerque, T.G., Costa, H.S., Food Chem. 2014, 154, 71-77.
[5] Silva, M.A., Albuquerque, T.G., Oliveira, M.B.P.P., Costa, H.S. Food Chem. 2017, https://doi.org/10.1016/j.foodchem.2017.11.046.
[6] Center for Drug Evaluation and Research, Reviewer Guidance: Validation of Chromatographic Methods. US FDA Federal Register, Rockville 1994.
[7] International Conference on Harmonization (ICH), Guidance for Industry Q2b: Validation of Analytical Procedures: Methodology, US FDA Federal Register, Rockville 1997.

Acknowledgements

This work was funded by INSA under the project "BIOCOMP" (2012DAN730). Tânia Gonçalves Albuquerque acknowledges the PhD fellowship (SFRH/BD/99718/2014) funded by the FCT, FSE and MEC.